Journal of Medicinal Plants and By-products (2013) 2: 205-208

Short communication

Essential Oil Content and Composition of Lemon Verbena (*Lippia citriodora* Kunth.) during Different Phenological Stages

Reza Shahhoseini[1], Arman Beyraghdar[1], Seyed-Razi Karimi[2] and Mohammad-Taghi Ebadi[1*]

[1]*Department of Horticulture, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran*
[2]*Department of Agricultural Mechanics, Tarbiat Modares University, Tehran, Iran*

Article History: Received: 19 February 2013/Accepted in revised form: 03 August 2013

Abstract

In this study, aerial parts of *Lippia citriodora* Kunth were harvested at three phenological stages (i.e. vegetative, full flowering and fruit set). The essential oil isolated by hydro distillation for 3 h using a Clevenger-type apparatus and the oil was analyzed by GC and GC-MS. The yields of oils (w/w%) in different stages was in the order of: full flowering (0.9%), vegetative (0.48%) and fruit set (0. 25%). In total, 13, 12 and 11 compounds of essential oil were identified in vegetative, full flowering and fruit set, respectively. The main compounds in three stages were geranial and neral. Geranial was highest at vegetative (33.7%) and lowest at full flowering (32.7%) stages. The highest and lowest neral content was observed at vegetative (26.1%) and fruit set (25.06%) stages, respectively.

Key words: Lemon verbena, *Lippia citriodora* Kunth, Phenological stages, Geranial, Neral, Verbenaceae

Introduction

Lippia citriodora Kunth, is a medicinal and aromatic plant belonging to Verbenaceae family. The leaves have a strong flavor and the typical smell lemon [1]. In South America, North Africa (Morocco) and Southern Europe, the leaves of *Lippia citriodora* are largely used as herbal tea for their aromatic, digestive and antispasmodic properties. The lemon verbena traditionally used for colds, fever and spasms as described by Duke [1]. Also Lemon verbena leaves are used to add a lemony flavor to fish and poultry dishes, vegetable marinades, salad dressings, jams, puddings, and beverages [2]. The essential oil from its leaves has antimicrobial activity [2,3]. Essential oil yield and composition depend on pedoclimatic conditions and developmental stage of plants. It is important accurately determine harvesting time for best yield and essential oil quality. In a study, Rode [4] reported that essential oil content in dried leaves of Lemon verbena was season and harvest dependent and varied from 0.81% to 1.19%. Several studies concluded that both essential oil yield and composition are highly dependent on plant ontogenesis, being the flowering stage as the optimal time point for highest essential oil yield and favorable essential oil composition [5,6]. Clark and Menary, in 1979, showed that and agronomic practices such as harvest stage [7] influence peppermint essential oil content and composition of peppermints. The phytochemical profile of the essential oils of *Lantana camara* L. (Verbenaceae) presented significant difference in function of the harvest [8]. Amiri *et al.* reported that highest essential oil yield from *Smyrnium cordifolium* Boiss. Plants were obtained at the ripen fruits stage, while the lowest yield was related to the harvest stage of pre-flowering [9]. The major compounds of essential oil from lemon verbena leaves are neral and geranial [10]. In another study, Argyropoulou, *et al.* in 2007, reported that the most important components of essential oil from lemon verbena at two developmental stages were geranial, neral and limonene that constituting 66.3% of the total essential oil content in May and increasing to 69% in September. However, their individual content values,

*Corresponding author: Department of Horticulture, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. P. O. Box: 14115-336.
E-mail Address: m.t.ebadi@gmail.com

changed for geranial and neral decreasing from 38.7 to 26.8% and from 24.5 to 21.8%, respectively, and for limonene increasing from 5.8 to 17.7% [11]. Koochaki and Teymoori, in 2012, reported that the highest amount of essential oil of *Lavandula angustifolia* Mill.will be achieved at the second harvesting stage of these plants [12].

Harvesting time is a critical operation in medicinal plants. Early harvesting probably leads to lower active substance and on the other hand late harvesting can leads to shattering of leaves, flowers and fruits [13,14]. The aim of the present investigation was to study the changes on the essential oil content and composition of *Lippia citriodora* during different phenological stages.

Material and Methods

Plant material

This study was carried out in research greenhouses of faculty of agriculture, Tarbiat Modares University, Iran, in 2009 growth season. For this experiment, greenhouse equipped with optical, ventilation, cooling, heating and irrigation systems. Healthy and equal cuttings with 30 cm length and 5 mm diameter were prepared. Cuttings were put in sand bed and rooted after 2 months. Rooted cuttings transferred to the main bed in greenhouse and cultivated in 50*50 cm rows. Before planting, to determine the nutritional status of the soil, project site soils were analyzed (Table 1). During the growth period, any kind of chemical inputs not used and weeds rogued mechanically. Aerial parts of plants were collected at the three phenological stages (vegetative, full flowering and fruit set) with 3 replications and dried in shaded area. Extraction of the essential oils

Shade dried samples (30 g, 3 times) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce oil. The oil was dried over anhydrous sodium sulfate and stored in sealed vial at low temperature (4 °C) before analysis.

Gas Chromatography analysis

Gas Chromatography analysis was performed on an Agilent technologist model Younglin Acm 600 equipped with a DB-5 fused silica column (30m×0.25mm×0.25µm). oven temperature was programmed from 50 ºC to 185 ºC at 3 ºC/min. injector and detector (FID) temperature were 290 ºC and helium was used as carrier gas (0.8 mL/min). Percentages were calculated by electronic integration of FID peak areas without the use of response factor correction. Essential oil samples were diluted with normal hexane and 0.1 ml was injected into the oven.

Gas Chromatography-Mass Spectrometry (GC/MS)

GC-MS analysis was carried out on an Agilent 6890 gas chromatograph fitted with a fused silica column HP-5MS (30m×0.25mm×0.25µm) capillary column. oven temperature was programmed from 50 ºC to 185 ºC at 3 ºC/min, and helium was used as carrier gas (0.8 mL/min), Mass spectra were obtained in an Agilent 5973 system operating in electron impact mode (EIMS) at 70 eV, coupled to an Agilent 6890 gas chromatograph fitted with a HP-5MS (30 m × 0.25 mm × 0.25 µm), using same injection procedure but oven temperature program was 220 ºC.

Essential oil content of *L. citriodora* at tree phenological stages including: vegetative, full flowering and fruit set; was given in Table 2. The highest (0.90%) and lowest (0.25%) essential oil content were found at full flowering and fruit set stages, respectively. Essential oil content at vegetative stage was 0.48%. In a study by Rode [4] content of essential oil of Lemon verbena was ranged between 0.81 to 1.19%.

Table 2 Essential oil content of *L. citriodora* during different phenological stages

Vegetative	Full Flowering	Fruit Set
0.48%	0.90%	0.25%

Results and Discussion

Differences between our results and this study can be explained by differences in the environmental conditions of regions under study which affect on essential oil content of *L. citriodora*.

The chemical composition of the essential oil during the vegetative stage is presented in Table 3. Thirteen compounds were identified.

Table 1 The results of the soil analysis

Soil texture	Clay (%)	Silt (%)	Sand (%)	EC (ds/m)	pH	OC (%)	$(Ca+Mg)^{+2}$ meq/L	Na meq/L	N (%)	P (mg/kg)	K (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	SAR
Loom	24	33	43	3.2	7.2	0.56	58.5	36.4	0.8	90	592	9	4.5	4.32	0.94	6.73

Table 3 Essential oil composition of *L. citriodora* during different phenological stages

Components	KI	Before Flowering	Full Bloom	Fruit Set
5-hepten-2-one, 6-methyl	985	4.6	5.2	4.6
Limonene	1031	7.5	7.6	4.8
(Z)-β-ocimene	1040	1.8	1.9	2.4
Neral	1240	26.1	26.0	25.1
Geranial	1270	33.3	32.7	32.9
δ-elemene	1339	1.0	-	-
Geranyl acetate	1383	1.8	1.6	1.8
α-cis bergamotene	1415	2.1	1.8	1.3
(Z)-β-farnesene	1443	2.6	1.8	-
α-acoradiene	1463	1.0	1.3	2.4
β- chamigrene	1475	2.7	1.8	-
(Z)-nerolidol	1534	1.2	-	1.3
Spathulenol	1576	-	1.6	7.1
Caryophyllene oxide	1581	2.9	-	2.9
Epi-cedrol	1611	-	0.8	-
Total (%)	-	88.6	84.1	86.6

Geranial (33.3%), neral (26.1%) and limonene (7.5%) were found to be the main components, constituting 66.9% of the total oil, followed by 5-Hepten-2-one, 6-methyl (4.6%), caryophyllene oxide (2.9%), β -chamigrene (2.7%), (Z)-β-farnesene (2.6%), (Z)-β-ocimene (1.8%) and geranyl acetate (1.8%).

The analysis of the essential oil in full flowering period gave 13 compounds (Table 3). Geranial (32.7%), neral (26.0%) and limonene (7.6%) were the most components. 5-Hepten-2-one, 6-methyl (5.9%), (Z)-β-ocimene (1.9%), β-chamigrene (1.8%) and (Z)-β-farnesene (1.8%) were found in minor percentages.

In fruit seed stage 11 compounds was found geranial (32.9%), neral (25.1%) and spathulenol (7.1%) were the most components. Limonene (4.8%) 5-Hepten-2-one, 6-methyl (4.5%), caryophyllene oxide (2.9%), (Z)-β-ocimene (2.4%) and α-acoradiene were found in minor percentages (Table 3) The main compounds in three stages were geranial and neral. Geranial was highest at vegetative (33.3%) and lowest at full flowering (32.7%) stages. The highest and lowest neral content was observed at vegetative (26.1%) and fruit set (25.1%) stages, respectively. These results were in agreement with those achieved by Argyropoulou, *et al*. in 2007, [11].

The content and composition of the essential oils of many medicinal and aromatic plants is greatly influenced by agronomic conditions, such as harvesting time Bylaite *et al*. [15]; Santos-Gomes and Fernandes-Ferreira, [16]; Sefidkon *et al*. [17]. Timing of the harvest is important with respect to oil yield and composition [13, 18,19] and with respect to the overall economics. Our results were in agreement with this phenomenon. Carnat, *et al*. [10] identified that major compounds of essential oil from lemon verbena leaves were neral and geranial. Also Argyropoulou, *et al*. [11] reported that main essential oil components of *Lippia* essential oil were neral and geranial, while their content varied between different harvest stages. These results were in consistency with our results. In our study the most component of essential oil were geranial, neral and limonene that are agreement with above study. In studies carried out by Argyropoulou *et al*. [11] about evaluation of lemon verbena (*Lippia citriodora*) oil composition and content during the two phenological stages, the most important components were geranial, neral and limonene that constituting 66.3% of the total essential. Our results were in agreement with this study. Thus the time of harvesting of this plant have a major effect on content and chemical composition of the essential oil. The flowering stage is the best time for harvesting the plant and obtaining the essential oil, because at this time the plant contains highest percent of the essential oil. Ruminska, in 1970, [20] declared that essential oil content of some essential oil bearing plants species were highest at full flowering stage.

In conclusion, our results showed that the variation in the *Lippia citriodora* oil may be linked to different phenological stages and harvesting times along with other parameters such as edaphic and climatic factors. These results clearly demonstrate the importance of harvesting stages either on essential oil yield or essential oil compositions. Hence, the harvest stage, as a point of view of commercial production of medicinal plants raw materials, plays an important role for being used as herbal medicines.

Acknowledgements

Authors thank the Tarbiat Modares University for financial support of this study.

References

1. Duke JA. Handbook of medicinal herbs, CRC Press, 1985. 896 pages.
2. Duarte MC, Figueira GM, Sartoratto A, Rehder V.L, Delarmelina C. Anti-Candida activity of Brazilian medicinal plants. J Ethnopharmacol. 2005;97:305-311.
3. Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLG. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Braz J Microbiol. 2004;35:275-280.
4. Rode J. Possibilities of *Lippia citriodora* Kunth. Cultivation in Slovenia. Acta Hort. 2000;523:61-64.
5. Rohloff J. Essential oil composition of sachalinmint from Norway detected by solid-phase microextraction and gas chromatography/ mass spectrometry analysis. J Agric Food Chem. 2002;50:1543-1547.
6. Rohloff J, Skagen EB, Steen AH, Beisvag T, Iversen TH. Essential oil composition of Norwegian peppermint (*Mentha piperita* L.) and sachalinmint (*Mentha sachalinensis*). Acta Agric Scand. 2000;50:161-168.
7. Clark RJ, Menary RC. The importance of harvest date and plant density on the yield and quality of Tasmanian peppermint oil. J Amer Soc Hort Sci. 1979;104:702-706.
8. Sousa OE, Colares AV, Fabiola FG, Campos AR, Lima, SG, Costa JGM. Rec Nat Prod. 2010;4:31-37.
9. Amiri H, Khavarinezhad R, Roostaeian A, Mshkatalsadat M.H. Research and developement in agronomy and horticulture. 2006;73:195-199.
10. Carnat A, Carnat AP, Fraisse D, Lamaison JL. The aromatic and polyphenolic composition of lemon verbena tea. Fitoterapia. 1999;70:44-49.
11. Argyropoulou C, Deferera D, Tarantilis PA, Fasseas C, Polissiou M. Chemical composition of the essential oil from leaves of *Lippia citriodora* H.B.K. (Verbenaceae) at two developmental stages. Biochem Syst Ecol. 2007;35:831-837.
12. Koochaki A, Teymoori M. Effect of irrigation round, fertilizers and harvesting stage on essential oil content and yield of three medicinal plants: *Lavandula angustifolia*, *Rosemarinus officinalis* and *Hyssopus officinalis* in Mashhad. Iranian J Field Crop Res. 2012;10:458-494.
13. Rohloff J, Dragland S, Mordal R, Iversen T.H. Effect of Harvest Time and Drying Method on Biomass Production, Essential Oil Yield, and Quality of Peppermint (*Mentha piperita* L.). J Agric Food Chem. 2005;53:4143-4148.
14. White JGH, Iskandar SH, Barnes MF. Peppermint: Effect of time of harvest on yield and quality of oil. New Zeal J Exp Agric. 1987;15:73-80.
15. Bylaite E, Venskutonis R, Roozen JP, Posthumus MA. Composition of essential oil of costmary [*Balsamita major* (L.) Desf.] at different growth phases. J Agric Food Chem. 2000;48:2409-2414.
16. Santos-Gomes P.C, Fernandes-Ferreira M. Organ and season dependent variation in the essential oil composition of *Salvia officinalis* L. cultivated at two different sites. J Agric Food Chem. 2001;49:2908-2916.
17. Sefidkon F, Abbasi K, Jamzad Z, Ahmadi Sh. The effect of distillation methods and stage of plant growth on the essential oil content and composition of *Satureja rechingeri* . Food Chem. 2007;100:1054-1058.
18. Clark RJ, Menary RC. The effect of two harvests per year on the yield and composition of Tasmanian peppermint oil (*Mentha piperita* L.). J Sci Food Agric. 2006;35:1191-1195.
19. Marotti M, Dellacecca V, Piccaglia R, Giovanelli E. Effect of harvesting stage on the yield and essential oil composition of peppermint (*Mentha piperita* L.). Acta Hort. 1993;344:370-379.
20. Ruminska A. Abhangigkeit zwischen der Entwicklungsphase und dem Ol- und Azulengehalt als auch dem Ernteertrag der Schafgarbe. Acta Agrobot. XXIII 1970.